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Modulation of Bacterial Host Phenotypes by Mycobacteriophage Pixie Gene Products

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Bacteriophage genes are being studied for their potential clinical use in phage therapy for antibiotic-resistant infections. With the support of the Howard Hughes Medical Institute, as part of the SEA-GENES network, we have amplified all 100 genes from Mycobacteriophage Pixie and cloned 88 genes for study in cytotoxicity assays. Genes were amplified from Pixie high titer lysate by PCR amplification, and the products were purified and ligated into a pExTra plasmid by isothermal assembly. Plasmids were transformed into 5-alpha F’Iq Escherichia coli, and the extracted plasmid DNA was electroporated into *Mycobacterium smegmatis* mc²155. Phenotypic assays were conducted by plating transformed *M. smegmatis* on agar containing anhydrotetracycline to induce the production of gene inserts. Cytotoxicity was determined by spotting serially diluted transformed *M. smegmatis* growth versus controls. Superinfection assays were conducted by inoculation of transformed or control *M. smegmatis* lawns with serially diluted bacteriophages to determine the efficiency of plating. Our study revealed 5 potentially cytotoxic genes: a putative major capsid protein, a holin protein downstream of the lysin A/B proteins, putative tyrosine integrase, and an uncharacterized gene adjacent to the immunity repressor. This information broadens our understanding of bacteriophage-bacteria interactions and the potential clinical use of cytotoxic genes to treat bacterial infections.